Photodynamic Action of Methylene Blue on Nicotine and Its Derivatives

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Introduction

It has previously been reported (1) that when an aqueous solution of *l*-nicotine was exposed to visible light in the presence of traces of methylene blue, rapid bleaching of the dye took place. It was concluded that the "light-excited" dye acted as a hydrogen acceptor and the *l*-nicotine as a hydrogen donor. In the presence of oxygen, the leuco dye was reoxidized and served continuously as a hydrogen acceptor. The work reported here is a detailed extension of our preliminary observation on the photodynamic action of methylene blue on nicotine and related compounds.

Anaerobic Reaction

Effect of pH

An aqueous solution of *l*-nicotine (2.5 ml.) containing 40 mg. adjusted to the desired pH and 2.5 ml. of citrate–phosphate buffer (2) of the corresponding pH were placed in Thunberg tubes. For obtaining pH 10 and 11, the buffer was adjusted with NaOH. Methylene blue solution (0.5 ml.) containing 0.2 mg. was measured into the side arms. After repeated evacuation and filling with nitrogen, the tubes were placed in a glass-walled water bath at 37°. A 150-w. "spotlight" lamp placed at a distance of 30 cm. served as a light source. After the dye was mixed with the reaction mixture, the time necessary for complete bleaching was measured. The entire operation was carried out in a dark room. The optimum pH for the bleaching was 9.0 (Table I).

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Effect of l-Nicotine Concentration at pH 9

To 2.5 ml. of 0.2 *M* solution of phosphate buffer of pH 9.0, 0.5 ml. of methylene blue (0.2 mg.) and 2.5 ml. of nicotine solution adjusted to pH 9.0 were added in varying concentrations. As Table I shows, the bleaching of methylene blue was accelerated by increased nicotine concentration up to about 10%. This might be due to the increased chances of collision between molecules of nicotine and the "light-excited" dye up to that concentration. Beyond that concentration there was no

TABLE I

Effects of pH, Concentration of Nicotine and of Methylene Blue, and Temperature on the Rate of Anaerobic Bleaching of Methylene Blue During Irradiation

pH		Nicotine concentration		Methylene blue concentration		Temperature	
Value	Bleaching time	%	Bleaching time	Methylene blue in 5.5 ml.	Bleaching time	°C.	Bleaching time
	sec.		*sec.*	*mg.*	*sec.*		*sec.*
3.0	>4 hr.	0.1	35	0.05	4.6	20	16.4
5.0	2880	0.8	16	.1	9.5	30	12.8
6.0	175	4.0	14	.2	22	40	13.0
7.0	57	6.0	11	.3	46	50	12.7
8.0	37	8.0	9	.4	70	60	12.6
9.0	13	10.0	8	.5	103	70	12.6
10.0	14	20.0	7	.75	237		
11.0	15	30.0	7	1.0	460		

decrease in bleaching time, probably owing to the limitation of available "light excited" dye molecules.

Effect of Methylene Blue Concentration at pH 9

Similar experiments were carried out with different concentrations of methylene blue and 40 mg. nicotine. The results (Table I) show that the bleaching time was fairly proportional to the methylene blue concentration up to 0.2 mg. Beyond that concentration, probably because of decreased light transmission, the bleaching rate decreased.

Effect of Temperature at pH 9

For these experiments 0.1 mg. of methylene blue and 40 mg. of nicotine were used. The results (Table I) show that the bleaching time

was practically not affected by the temperature. The temperature coefficient between 20° and 70° was only 1.30, indicating a true photochemical reaction.

Effect of Wavelength

According to the Grotthus-Draper absorption law, only light that is absorbed can produce chemical changes. The active radiation is limited to the wavelengths absorbed by the sensitizer. To apply this law to our system, glass filters (Corning) of different transmission range were used. A parallel beam of light from a 100-w. Mazda projection lamp was passed through the light filter into the Thunberg tubes. The reaction mixture at pH 9.0 was the same as that used before. The tubes were placed in a glass-walled water bath at 37° at a distance of 10 cm. from the glass filter. Six filters covered the range of 340–700 mμ. At wavelengths from 340 to 640 mμ, there was no bleaching in 2 hr., but at 660 mμ bleaching occurred in 9 min. This active region corresponds closely to the known absorption maximum of methylene blue at 670 mμ.

To study the effect of ultraviolet light, the reaction mixture was placed in a quartz container of 12 mm. thickness and exposed to the radiation of a quartz lamp (Hanovia, 60 cycles, 125 w., single phase) from a distance of 10 cm. for 2 hr. There was no reaction.

These reactions in the absence of oxygen can be considered essentially a dehydrogenation of *l*-nicotine, in which the dye acted as a hydrogen acceptor. With higher concentration of dye, the leuco methylene blue formed in this process could be easily demonstrated as a crystalline precipitate during the irradiation. The dehydrogenation of nicotine in this process should result in unsaturation, which if conjugated, should manifest itself in the ultraviolet absorption spectrum. Our attempt in this direction has failed so far, because of the difficulty of removing the leuco dye.

Aerobic Reaction

When the irradiation was carried out in oxygen, the leuco dye formed was reoxidized and hence could serve repeatedly as a hydrogen acceptor. The oxygen required for the reoxidation thus served as an indicator of the rate of the reaction.

Equipment

An adaptation of the manometric technique of Warburg was used. White light of high intensity was supplied by a battery of four 150-w. "spotlight" lamps connected in parallel over a length of 58 cm. and placed 38 cm. from the glass wall of the water bath. The light was reflected on the bottom of the vessel by a mirror placed at an

angle of 45° under the respirometers. With this arrangement, an even intensity of light was produced, as indicated by the same oxygen uptake in all the Warburg vessels in identical experiments. The reaction temperature was maintained at 37°C. unless otherwise stated. To prevent overheating by the light, a water-cooled glass tube was placed in the water bath; the flow of water was regulated by a solenoid valve. The vessels were shaken at a rate of 120 strokes/min. at an amplitude of 5 cm. An increase of this speed did not increase the rate of oxygen uptake. Because of the large oxygen uptake, mercury was used as a manometer fluid.

Effect of pH

In the main chamber of the Warburg vessels were placed 1.0 ml. of an aqueous solution of *l*-nicotine containing 40 mg. and adjusted with HCl to the proper pH, and 0.5 ml. of 0.2 *M* citrate–phosphate buffer of pH 2.50, 4.10, 6.0, 6.90, 7.88, 8.70, or 9.30. To obtain pH 9.3, the buffer was adjusted with alkali. Methylene blue solution (0.5 ml.) containing

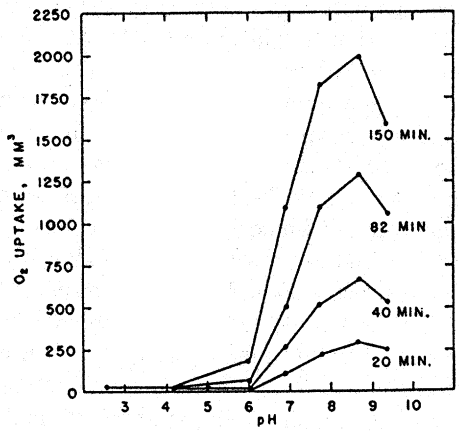

FIG. 1. Effect of pH on the photodynamic action of methylene blue on nicotine.

0.1 mg. was introduced into the side arm. The results (Fig. 1) show a pH optimum of about 8.7 for the oxygen uptake. A similar optimum was found (Table I) for the anaerobic bleaching.

Effect of Temperature

The reactants consisted of 1.0 ml. of *l*-nicotine solution containing 8.1 mg., 0.5 ml. of 0.2 *M* phosphate buffer of pH 8.7, and 0.5 ml. of

methylene blue solution containing 0.1 mg. Irradiation was carried out at 30°, 40°, and 50°C. Figure 2 shows the results.

In contrast with the results in Table I, the reaction rate was distinctly accelerated by increased temperature, indicating the participation of a dark reaction in addition to the previously observed light-sensitive reaction. The reaction ceased after 1 mole of *l*-nicotine had taken up 1 mole of oxygen.

Presence and Absence of Light

To 1.0 ml. of *l*-nicotine solution containing 20 mg., 0.5 ml. of 0.2 *M* phosphate buffer of pH 8.7, and 0.5 ml. of methylene blue solution

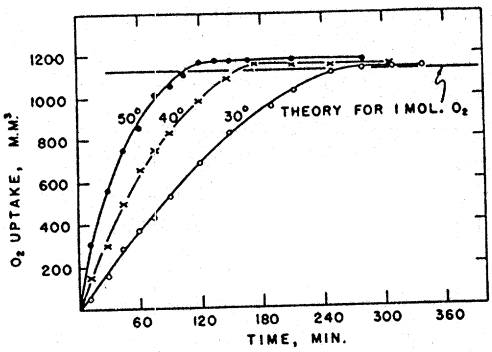

FIG. 2. Effect of temperature on the photodynamic action of methylene blue on nicotine. The theoretical oxygen uptake calculated for 1 mole of substrate.

containing 0.1 mg. were added. The results (Fig. 3) demonstrate that during irradiation a rapid oxygen uptake took place, but during the intermittent dark period there was only a slight uptake.

Is CO_2 Evolved?

Experimental conditions were the same as those described for the preceding experiment. The two-vessel method (3) was used; the center well of one of the vessels contained KOH. To avoid possible retention of CO_2 by the phosphate buffer, acid was added after completion of the reaction. These experiments gave no indication of evolution of CO_2.

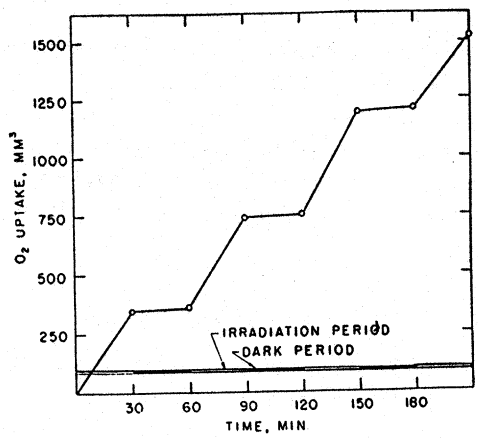

FIG. 3. Action of methylene blue on nicotine in the presence and absence of light.

Proportionality Between Oxygen Uptake and Concentration of l-Nicotine

When the amount of nicotine was varied but otherwise the experimental conditions were kept the same as for the preceding experiment, the amount of oxygen uptake was strictly proportional to the nicotine concentration (Fig. 4). The oxygen uptake in each instance ceased when

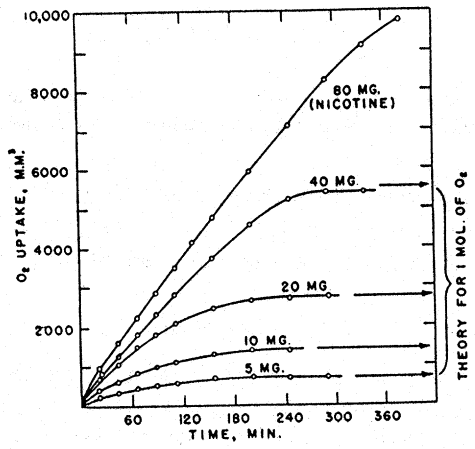

FIG. 4. Photodynamic action of methylene blue on nicotine at different concentrations. The theoretical oxygen uptake calculated for 1 mole of substrate.

1 mole of oxygen was taken up by 1 mole of *l*-nicotine. At a nicotine concentration of 80 mg., the reaction was not completed, but the oxygen uptake had almost reached the theoretical value.

Action of Various Thiazine Dyes on l-Nicotine

To 1.0 ml. of *l*-nicotine solution containing 16.2 mg. were added 0.5 ml. of 0.1 *M* phosphate buffer of pH 8.7 and 0.5 ml. of dye solution containing 0.1 mg. of methylene blue, thionine, new methylene blue, toluidine blue, or methyl green. The results, presented in Fig. 5, show

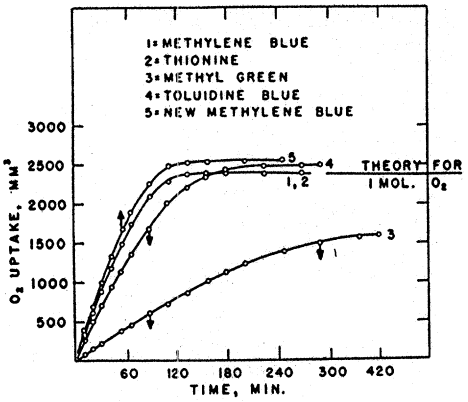

FIG. 5. Photodynamic action of various thiazine dyes on nicotine. The theoretical oxygen uptake calculated for 1 mole of substrate.

that with the exception of methyl green the dyes are comparable to methylene blue in their photodynamic action. Owing to the photochemical destruction of these dyes, however, they had to be renewed to maintain a steady rate of oxygen uptake. The stage at which dye was added is indicated in Fig. 5 by arrows. Because of its fastness to light, methylene blue is a superior dye in comparison with the others, and was therefore used throughout our experiments.

Effect of Concentration of Methylene Blue

Twenty mg. of *l*-nicotine was irradiated as described in the preceding experiment but in the presence of different amounts of dye. Figure 6 shows the results. The optimum concentration of methylene blue was

between 0.05 and 0.2 mg. Below and above these concentrations, the rate of oxygen uptake decreased. Slower reaction at low methylene blue concentrations may also originate from the smaller number of excited molecules per unit of time, whereas at the higher concentrations there was a decreased transmission of light.

Action on Various Nicotine Derivatives

To gain some information on the site of the nicotine molecule which is brought into the reaction by means of the photosensitized methylene blue molecule, various nicotine derivatives were studied. In each case, an amount equivalent to 16.2 mg. of *l*-nicotine was used, and 0.5 ml. of 0.2 *M* phosphate buffer of 8.7 pH and 0.1 mg. of methylene blue were added. The total volume was 2 ml.

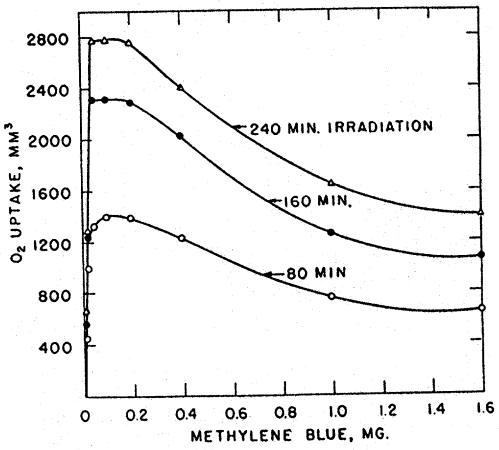

FIG. 6. Photodynamic action of different amounts of methylene blue on nicotine.

The results (Fig. 7) demonstrate the importance of the *N*-methylpyrrolidine portion of the nicotine molecule, since this compound is photoöxidized at the same rate and to the same extent as *l*-nicotine itself. The results also indicate that the entire oxygen uptake is confined to this portion of the *l*-nicotine molecule.

Comparison of *l*-nicotine with nornicotine, and *N*-methylpyrrolidine with pyrrolidine, brings out the additional importance of the tertiary amino nitrogen. If the oxygen uptake of *l*-proline is compared with that

of hygric acid, and also if the reactivity of piperidine is compared with that of *N*-methylpiperidine, a similar increase in photochemical reactivity is observed on formation of a tertiary amino group.

Pyridine, β-picoline, β-vinylpyridine, nicotinic acid, nicotinamide, myosmine, and nicotine oxide were essentially unaffected in this reaction. The inactivity of these compounds indicates that the pyridine portion of the nicotine molecule did not participate in the photoöxidation of *l*-nicotine.

Action on Aliphatic Amines

Figure 8 presents our findings with the various methyl-, ethyl-, propyl-, and butylamines. The amounts used were: for the methyl

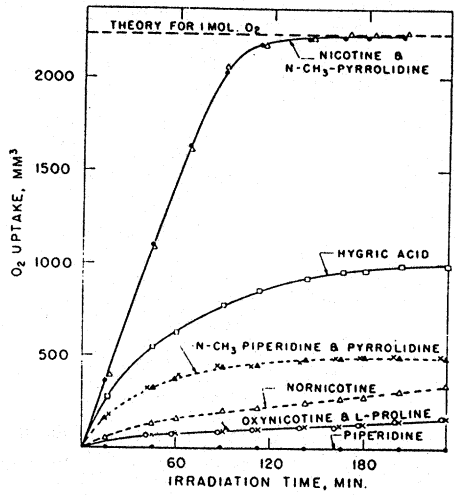

FIG. 7. Photodynamic action of methylene blue on nicotine and its derivatives. The theoretical oxygen uptake calculated for 1 mole of substrate.

derivatives, equivalent to 5.9 mg. of trimethylamine; for the ethanol derivatives, equivalent to 14.9 mg. of triethanolamine; for the propyl derivatives, equivalent to 14.3 mg. of tripropylamine, dissolved in 50% ethanol; for the butyl derivatives, equivalent to 18.5 mg. of tributylamine, dissolved in 95% ethanol. The results (Fig. 8) confirm the high reactivity of the tertiary amino group during the photodynamic action of methylene blue, and supports our claim that this group of the pyr-

rolidine ring in the nicotine molecule is of determinate importance. The interpretation of the results with aliphatic amines, however, has to await further investigations.

In the case of trimethylamine, a larger-scale experiment was carried out to isolate the reaction product. Ten grams of trimethylamine and 75 mg. of methylene blue were dissolved in 750 ml. of water and irradiated for 11 hr. (as described later for *l*-nicotine). During that time, at 27° and atmospheric pressure, 1950 ml. of oxygen was taken up. The

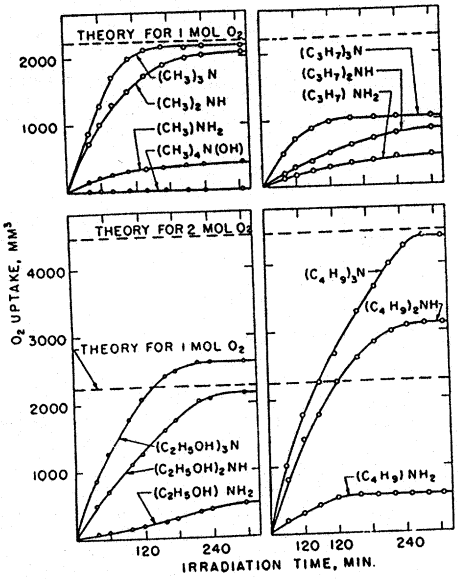

FIG. 8. Photodynamic action of methylene blue on aliphatic amines. The theoretical oxygen uptake calculated for 1 mole of substrate.

solution was treated with activated carbon to remove the dye and then evaporated in vacuum at 45°. An oily residue (5.2 g.) insoluble in ether was obtained. The ether-extracted residue was dissolved in absolute alcohol and acidified, and on cooling formed a white crystalline precipitate. After three recrystallizations, 2.3 g. of the pure compound was obtained, with a melting point of 208.3–210.6° (corr.). A mixed melting point with trimethylamine oxide hydrochloride gave no depression.

Analysis. Calcd. for $C_3H_{10}NOCl$: C, 32.28%; H, 8.97%; N, 12.56%; Cl, 31.78%; found: C, 32.58%; H, 8.88%; N, 12.54%; Cl, 31.46%.

Thus, the irradiation product of trimethylamine was trimethylamine oxide.

Inasmuch as trimethylamine took up 1 mole of oxygen (Fig. 8), whereas the resulting irradiation product accounted for only ½ mole, it is possible that trimethylamine oxide represents only a breakdown product of a labile end product. The result, however, supports the view advanced later in this paper that one of the oxygen atoms in the irradiated *l*-nicotine is an amine oxide.

Action on Alkaloids

The photochemical reactivity of the tertiary amino group was further demonstrated on the following plant alkaloids, all of which contain a tertiary amino group: homatropine, atropine, physostigmine, hyocyamine, scopolamine, sparteine, lobeline, arecoline, pilocarpine, hydrastine, and hordenine. They could be easily photoöxidized in the presence of methylene blue and visible light.

Action on Various Nicotine Homologs

To study influence of substitution on the pyrrolidine nitrogen of nicotine, the *N*-methyl group was substituted by octyl and octadecyl radicals. Also included was α-aminonicotine. The amount of each compound used was equivalent to 16.2 mg. of nicotine. Other conditions

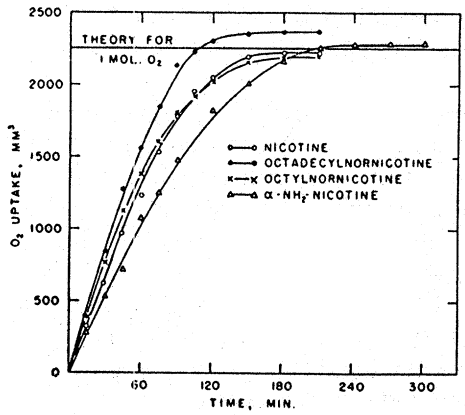

FIG. 9. Photodynamic action of methylene blue on nicotine homologs. The theoretical oxygen uptake calculated for 1 mole of substrate.

were the same as those described for the experiment represented by Fig. 7. The results (Fig. 9) show that the replacement of the *N*-methyl group of nicotine by octyl or octadecyl radicals had practically no effect on oxygen absorption. α-Aminonicotine behaved similarly, not showing any additional oxygen uptake. This was expected, inasmuch as it had been shown previously that the pyridine portion of the nicotine molecule was not involved in the photoöxidation.

Action on l- and dl-Nicotine

To study the possible effect of optical isomerism, the action on *l*-nicotine and on *dl*-nicotine was compared. *dl*-Nicotine was prepared by methylation (4) of *dl*-nornicotine. The amount of each isomer used was 16.2 mg. Optical isomers had no effect on the photoöxidation; both forms were oxidized at the same rate and to the same extent.

Peroxide Formation

In view of Gaffron's work (5) concerning the photodynamic action of chlorophyll on propylamine, attempts were made to demonstrate the formation of peroxide during the photochemical oxidation of nicotine. Iodine titration and decomposition of any peroxide by manganese dioxide, platinum, and palladium catalysts failed to demonstrate the existence of such a group. Gaffron (5) found that the primary amines were photochemically highly reactive, whereas the corresponding secondary and tertiary amines were inactive. The different results in the experiments reported here might be due to the different experimental conditions, such as the use of solvent, and in the different sensitizer employed.

Inasmuch as the first step in the photodynamic action of methylene blue on nicotine is a transfer of hydrogen from nicotine to the dye, the subsequent reoxidation of the leuco compound by atmospheric oxygen should lead to hydrogen peroxide formation, as demonstrated by Wieland and Franke (6) and Macrae (7). Addition of a highly active catalase preparation to the nicotine–methylene blue system during or after irradiation failed to produce any evidence of formation of an intermediate hydrogen peroxide. During irradiation, however, rapid inactivation of the enzyme took place.

Our failure to demonstrate the formation of hydrogen peroxide when the catalase was added after completion of the photoöxidation of nico-

tine pointed to two possibilities: either no hydrogen peroxide was formed, or if it took place, it was instantly taken up by the reaction product of nicotine. To decide this point, a search was made for compounds that had sufficiently high affinity for hydrogen peroxide to compete with the as yet undefined intermediate of nicotine. Since Sevag (8,9) and Fujita and Kodama (10) had found that pyruvic acid reacts more quickly with nascent hydrogen peroxide than catalase, this compound was selected. According to Wieland and Franke (6), a catalase-resistant peroxide of pyruvic acid is formed, which is subsequently decomposed into 1 mole of acetic acid, 1 of CO_2, and 1 of water.

The photodynamic action of methylene blue on nicotine was studied in the presence of sodium pyruvate. The sodium pyruvate was prepared according to Robertson (11). To 16.2 mg. *l*-nicotine were added 11.1 mg. of sodium pyruvate (equivalent to the *l*-nicotine), 0.5 ml. of 0.2 *M* phosphate buffer of 8.7 pH, and 0.1 mg. methylene blue. The total volume was 2.0 ml.

TABLE II

Photodynamic Action of Methylene Blue on Nicotine in the Presence of Na Pyruvate

	Nicotine and methylene blue	Nicotine, methylene blue, and sodium pyruvate	Sodium pyruvate and methylene blue	Nicotine and sodium pyruvate	Sodium pyruvate
Moles of O_2 taken up	0.99	2.12	0.00	0.00	0.00
Moles of CO_2 evolved	0.00	1.04	0.00	0.00	0.00

As Table II shows, addition of equimolar amounts of sodium pyruvate to *l*-nicotine and methylene blue resulted in the formation of 1 mole of CO_2. Since CO_2 was not observed in any of the controls, it must have been produced by oxidation of sodium pyruvate by an intermediate oxidizing agent formed during the photodynamic action of methylene blue on *l*-nicotine. In view of Wieland and Franke's work (6), which clearly showed that the reoxidation of leuco methylene blue by molecular oxygen (this also occurs in our reaction) was accompanied by formation of hydrogen peroxide, it would appear that in the present case, also, this intermediate oxidizing agent is hydrogen peroxide.

An increase of sodium pyruvate above 1 equiv. resulted in the formation of but 1 mole of CO_2, indicating the intermediate formation of only 1 mole of hydrogen peroxide.

Experiments with sodium pyruvate and molecular hydrogen peroxide by the Warburg technique showed that even here the oxidation of sodium pyruvate was completed in about 10 min., with the evolution of only 1 mole of CO_2.

Formation of sodium acetate from sodium pyruvate, as required by the action of hydrogen peroxide, was also demonstrated. For this purpose, a larger-scale experiment was set up; 4 g. of *l*-nicotine, 2.44 g. of sodium pyruvate, and 40 mg. of methylene blue in 400 ml. of water were used. Irradiation was carried out at 37° until no further oxygen uptake was observed (about 8 hr.). The reaction mixture was then treated with active carbon to remove the dye, filtered, and the filtrate was evaporated to dryness in vacuum at 45°. The anhydrous residue was extracted with chloroform, and the white crystalline residue was identified as sodium acetate by the anilide (12). It melted at 113.8°. When it was mixed with a known sample of anilide of acetic acid, the melting point of the mixture was 113.6°.

Table II also shows that the addition of sodium pyruvate increased the oxygen uptake to 2 moles of oxygen, as contrasted with 1 mole when *l*-nicotine was photoöxidized alone. It appears that if formation of the intermediate hydrogen peroxide is eliminated, the resulting *l*-nicotine transformation product becomes susceptible to further oxidation, whereas in the absence of sodium pyruvate, as will be shown later, the hydrogen peroxide formed adds to the nicotine product, and makes it resistant to further oxidation.

Chemical Characterization of the l-Nicotine Irradiation Product

For this purpose larger-scale experiments were required. A 3-l. Fernbach flask, equipped with a mercury-seal stirrer, was placed in a cylindrical glass water bath at 37°. A 300-w. "spotlight" lamp placed 5 cm. under the water bath was the light source. The reaction vessel was connected with an oxygen reservoir, and the oxygen taken up during irradiation was replaced by introducing a measured amount of water into the reservoir. A mercury-filled manometer was attached to the reaction vessel. As a rule, 20 g. of *l*-nicotine and 100 mg. of methylene blue dissolved in 750 ml. of water were placed in the reaction vessel. After the reaction vessel was flushed with oxygen and connected with the oxygen reservoir, the light was turned on. In general, about 12–14 hr. of irradiation was required to complete the oxidation of 20 g. of *l*-nicotine. After completion of the oxidation, the methylene blue was

removed. The pH of the reaction mixture had dropped during the irradiation from 10.8 to 6.70, most likely because of formation of amine oxide. The clear yellowish filtrate was then evaporated in vacuum at 45°, and the oily residue was extracted repeatedly with ether to remove any traces of unreacted *l*-nicotine. The reaction product was insoluble in ether, benzene, carbon tetrachloride, and petroleum ether; it was soluble in water, alcohol, and chloroform. The specific optical rotation in 95% alcohol was $[\alpha]_{25}^{D} = -35.2$. A reproduction of this value with other preparations, however, was handicapped by the low light transmission of even more dilute solutions.

Analysis. Calcd. for $C_{10}H_{14}N_2O_2$: C, 61.85%; H, 7.21%; N, 14.43%; found: C, 62.69%; H, 7.21%; N, 14.08%. Extensive tests for aldehyde, keto, peroxide, carboxylic, and alcoholic groups were negative.

On reduction of the irradiation product of nicotine with zinc and acetic acid in alcoholic solution (13), 50–65% of the theoretical *l*-nicotine was recovered, although in our preliminary note (1), 85–100% recovery was reported.

Determination of the molecular weight of the irradiation product by the boiling point method in 95% ethyl alcohol gave a value of 378. Inasmuch as the minimum molecular weight calculated from the elementary analysis was 192, the experimentally found value indicates formation of a dimer. Calcd. for $(C_{10}H_{14}N_2O_2)_2$: 384; found: 378.

The ultraviolet absorption spectrum of the product did not differ from that of *l*-nicotine (14), indicating the absence of any formation of conjugated double bonds.

When the pH of a water solution of the irradiated *l*-nicotine was adjusted to 11, and the solution was steam distilled, 48.4% of the theoretical amount of *l*-nicotine, determined by titration, was found in the distillate. It was identified by the melting point and mixed melting point of the picrate (m. p. 222.3–223.0°; mixed m. p. 222.2–223.2°).

In 1892 Pinner and Wolffenstein observed (15) that if nicotine oxide was refluxed with concentrated hydrochloric acid, then alkalized and steam distilled, a crystalline compound could be isolated from the acidified steam distillate. They named the compound pseudonicotine oxide. The structure of the compound was unsolved for 58 years, when Haines and Eisner (16) identified it as *N*-methylmyosmine. The hydrochloric acid salt of this compound exists in the keto form, with an open chain system; in making the free base, a ring closure takes place resulting in formation of *N*-methylmyosmine.

The irradiation product of *l*-nicotine was subjected to a similar treatment; 15.5 g. of the irradiated *l*-nicotine was refluxed for 10 hr. with 120 ml. of concentrated hydrochloric acid. The reaction mixture was made alkaline and steam distilled. The distillate was then acidified with HCl and evaporated in vacuum at 50°. The crystalline residue weighed 6.3 g. When recrystallized from absolute alcohol, a white crystalline product was obtained with a melting point of 195.2–196.8°. When it was mixed with a crystalline product obtained from nicotine oxide, the melting point of the mixture was 195.8–196.8°.

Analysis. Calcd. for $C_{10}H_{14}N_2O \cdot 2HCl$: C, 47.82%; H, 6.41%; N, 11.14%; Cl, 28.23%; found: C, 47.13%; H, 6.59%; N, 10.93%; Cl, 27.93%.

Inasmuch as both nicotine oxide and the irradiated *l*-nicotine resulted in the same product, it is probable that both compounds have something in common, namely, an amine–oxide group. A similar shift of the amine–oxide into a keto position was observed by Haworth and Perkins (17) during their synthesis of cryptopine and protopine.

The irradiation product was not homogeneous. When 1.72 g. was dissolved in 25 ml. of water at pH 6.7, and exhaustively extracted with chloroform, in a liquid–liquid extractor, 85.44% of the total amount was soluble. No difference could be observed between the ultraviolet absorption spectrum of the chloroform-soluble fraction and that of the chloroform-insoluble fraction. Both were identical with the spectrum of the original irradiated *l*-nicotine, which, in turn, was identical with that of *l*-nicotine. The nitrogen content of the original irradiated *l*-nicotine was 14.23%; that of the chloroform-soluble fraction was 14.28%; and that of the chloroform-insoluble fraction, 14.25%. The identical ultraviolet absorption spectra and identical nitrogen values indicate that irradiated *l*-nicotine consists of at least two fractions, closely related and probably isomeric.

Discussion

Published reports on the photodynamic action of dyes are too numerous to be discussed here; we shall refer only to the extensive reviews of Blum (18) and Arnow (19) on this subject. Studies on the photooxidation of nicotine (20–23), however, were confined to the use of ultraviolet radiation, which was shown to be nonspecific, resulting in a far-reaching destruction of the nicotine molecule. The present work

describes a photodynamic action of visible light and methylene blue on *l*-nicotine that proceeds rapidly and reaches a definite end point.

The first step in this reaction involves, under the influence of sensitized light, the transfer of hydrogen from *l*-nicotine to methylene blue, which acts as a hydrogen acceptor. This phase represents a true photochemical reaction. The reduced dye is then reoxidized by atmospheric oxygen and serves continuoulsly as a hydrogen acceptor. Our experiments make it reasonable to assume that during the reoxidation of the leuco dye, as already established by Wieland and Franke (6), nascent hydrogen peroxide is formed, which, in turn, reacts with the irradiation product of nicotine.

We propose the following over-all equations:

$$\text{I. } 2C_{10}H_{14}N_2 + 2M\text{—}B^{2} \underset{\text{dark}}{\overset{\text{light}}{\rightleftharpoons}} 2C_{10}H_{12}N_2 + 2M\text{—}B(H_2).^{3}$$

$$\text{II. } 2M\text{—}B(H_2) + 2O_2 \dashrightarrow 2M\text{—}B + 2H_2O_2.$$

$$\text{III. } 2C_{10}H_{12}N_2 + 2H_2O_2 \dashrightarrow (C_{10}H_{14}N_2O_2)_2.$$

A similar concept was advanced recently by Galston (24) on the photodynamic action of riboflavin.

Reaction I represents the light reaction that is insensitive to temperature and can be partially reversed in the dark. Reactions II and III can be considered dark reactions, inasmuch as they can be increased by higher temperature. Although the chemical structure of the *l*-nicotine irradiation product is still unsolved, apparently it is a dimer, and one of the oxygen atoms in the molecule is present as an amine oxide of the pyrrolidine nitrogen. The position of the second oxygen atom is not known, although the possibility of formation of hydrate is not excluded. The compound does not appear to be homogeneous, and could consist of two or perhaps more components, which might be isomers.

During the fermentation of cigar leaf tobacco Frankenburg (25) found a similar, if not the same, conversion product of nicotine. It is suggested that during the fermentation a similar reaction takes place, in which the energy supplied by light in our system is replaced by the catalytic action of intracellular enzymes of the leaf, or perhaps of the bacterial flora, or the interaction of both.

[2] Methylene blue.

[3] Leuco methylene blue.

It is tempting to speculate that nicotine might play a role in the metabolism of the tobacco leaf, acting as a hydrogen-transferring agent with chlorophyll as a sensitizer; but this concept has to await evidence.

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Summary

Under anaerobic conditions, the photodynamic action of methylene blue on nicotine is manifested by rapid bleaching of the dye. The "light-excited" dye acts as a hydrogen acceptor, and the nicotine as a hydrogen donor. This hydrogen transfer is a true light reaction.

Under aerobic conditions, the reduced dye is reoxidized and thus acts repeatedly as a hydrogen acceptor. The oxygen uptake ceases after 1 molecule of oxygen per mole of nicotine is taken up. This phase of the reaction is sensitive to temperature.

There is evidence that hydrogen peroxide is formed during the reoxidation of the reduced dye.

Results of experiments with various nicotine derivatives and aliphatic amines indicate that the reaction centers around the tertiary pyrrolidine amino group of the nicotine molecule, involving formation of an amine oxide, whereas the pyridine portion of the molecule does not participate in the reaction.

Possible over-all equations are proposed for this oxidation of nicotine.

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